EPA/OPP MICROBIOLOGY LABORATORY ESC, Ft. Meade, MD

Standard Operating Procedure for Calibration of Kimble Class A Burets

SOP Number: EQ-06-03

Date Revised: 07-12-05

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SOP No. EQ-06-03 Date Revised 07-12-05 Page 1 of 8

TABLE OF CONTENTS

	<u>Contents</u>	Page Number
1.0	SCOPE AND APPLICATION	2
2.0	DEFINITIONS	2
3.0	HEALTH AND SAFETY	2
4.0	CAUTIONS	2
5.0	INTERFERENCES.	2
6.0	PERSONNEL QUALIFICATIONS	2
7.0	SPECIAL APPARATUS AND MATERIALS	2
8.0	INSTRUMENT OR METHOD CALIBRATION	3
9.0	SAMPLE HANDLING AND STORAGE	3
10.0	PROCEDURE AND ANALYSIS	3
11.0	DATA ANALYSIS/CALCULATIONS	3
12.0	DATA MANAGEMENT/RECORDS MANAGEMENT	5
13.0	QUALITY CONTROL	5
14.0	NONCONFORMANCE AND CORRECTIVE ACTION	6
15.0	REFERENCES	6
16.0	EODMS AND DATA SHEETS	6

1.0 <u>SCOPE AND APPLICATION</u>:

1.1 This protocol describes the calibration method for 10 mL and 50 mL Kimble Class A burets. The calibration is done on an annual basis.

2.0 DEFINITIONS:

- 2.1 Calibration = The determination of the difference between the volume dispensed and the expected volume.
- Adjustment = The correction of the difference between the measured value and the expected volume of the liquid displaced.
- 2.3 NIST= National Institute of Standards and Technology.
- 3.0 <u>HEALTH AND SAFETY</u>: Not applicable

4.0 CAUTIONS:

- 4.1 The water used for calibration should be weighed immediately after being dispensed to avoid evaporation.
- 4.2 Burets should be inspected for chips and cracks prior to use and prior to calibration.

5.0 INTERFERENCES:

5.1 It is important that the volumes of water are dispensed precisely, otherwise the entire calibration process can be impacted.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP.

7.0 <u>SPECIAL APPARATUS AND MATERIALS</u>:

- 7.1 Kimble Class A buret (10 mL) with 0.1 mL increments.
- 7.2 Kimble Class A buret (50 mL) with 1.0 mL increments.
- 7.3 Sartorius Basic Plus Model BP 211D (Serial Number 80904707): Weighs 0 to 40 / 80 / 210 g, reads to 0.00001 g or .01 mg, reproducibility $\leq 0.02 / 0.05 / 0.1$ mg (Manufacturer's Claims).
- 7.4 Sartorius Master^{Pro} Series Model LP 420 (Serial Number 81107148): Weighs 0 to 420 g, reads to 0.01 g or 10 mg, reproducibility ≤ 0.01 g (Manufacturer's

SOP No. EQ-06-03 Date Revised 07-12-05 Page 3 of 8

Claims).

7.5 Microsoft Excel Spreadsheet (2003)

8.0 INSTRUMENT OR METHOD CALIBRATION:

- 8.1 The weigh balances are calibrated annually by a professional calibration service and are checked quarterly for accuracy using a reference weight set certified by NIST (see SOP EQ-03, Weigh Balances).
- 9.0 <u>SAMPLE HANDLING AND STORAGE</u>: Not applicable

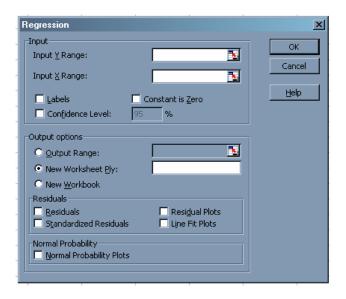
10.0 PROCEDURE AND ANALYSIS:

- 10.1 Wash and rinse the buret with de-ionized water. Place a clean 50 mL beaker on the balance and tare it. Fill the buret with room temperature de-ionized water and adjust the level of the water to the zero mark while allowing the rest of the buret to become filled. Remove any air bubbles. Record the temperature of the water on the Calibration of Kimble Class A Buret Form (see 16.1).
- 10.2 For the 10 mL buret, dispense into the pre-tared beaker 5 serial aliquots (2 mL each) of water from the filled buret. Weigh the pre-tared beaker after the addition of each 2 mL aliquot. Record results on the Calibration of Kimble Class A Buret Form (see 16.1).
- 10.3 For the 50 mL buret, dispense into the pre-tared beaker 5 serial aliquots (10 mL each) of water from the filled buret. Weigh the pre-tared beaker after the addition of each 10 mL aliquot. Record results on the Calibration of Kimble Class A Buret Form (see 16.1).
- 10.4 Weights are plotted against the independent variable of the volume reading on the buret using Microsoft Excel.

11.0 DATA ANALYSIS/CALCULATIONS:

- 11.1 Simple linear regression is calculated by Microsoft Excel.
 - 11.1.1 For the 10 mL buret, enter X values in column A of the spreadsheet and Y values in column B.
 - 11.1.2 In Microsoft Excel, select Tools \rightarrow Data Analysis \rightarrow Regression \rightarrow OK.

11.1.3 Pop-up menu:



11.1.4 In the pop-up menu under *Input*:

- Click once in the blank cell to the right of *Input Y Range*, and then highlight the column of 5 y-values in the spreadsheet.
- Click once in the blank cell to the right of *Input X Range*, and then highlight the column of 5 x-values in the spreadsheet.

11.1.5 In the same pop-up menu under *Output*:

- Select the *Output Range* option.
- Click once in the blank cell to the right of <u>Output Range</u>, and then highlight a single cell beneath the "Vol. X" column in the spreadsheet.

11.1.6 Select OK.

11.1.7 Summary output:

SUMMARY OUT	PUT							
Regression S	tati stics							
Multiple R	0.999994087							
R Square	0.999988173							
Adjusted R Square	0.999984231							
Standard Error	0.012564167							
Observations	5							
ANOVA	df	SS	MS	F	Significance F			
Regression	1	40.04241124		253660.4849	1.72618E-08			
Residual	3		0.000157858	255000.4045	1.720102-00			
Total	4	40.04288481						
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0.006658	0.01317741	0.505258638	0.648159495	-0.035278398	0.048594398	-0.035278398	0.048594398
X Variable 1	1.00053	0.001986569	503.647183	1.72618E-08	0.99420785	1.00685215	0.99420785	1.00685215

- 11.1.8 Repeat steps 11.1.1 through 11.1.7 for the 50 mL buret.
- 11.2 The corrected value, or adjustment, for any volume (y) dispensed by the buret is obtained by inserting the volume reading (in mL) on the buret into the following equation:

y = mx + b; where y = corrected volume, m = slope (x variable 1 coefficient), x = volume reading on buret, and b = y-intercept (intercept coefficient). The numbers are rounded to the nearest tenth of an mL.

11.2.1 A label displaying the date of calibration and corrected value (even if it is zero) for any volume dispensed by the buret is placed around the top of the buret.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly and in indelible ink on the Calibration of Kimble Class A Buret Form. The accompanying spreadsheet will be archived with the raw data sheet. Completed forms are archived in notebooks kept in secured file cabinets in the file room D 217. Only authorized personnel have access to the secured files. Archived data are subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The calibration of burets is performed annually and the information is documented on the appropriate record form(s) (see 16.1).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

- 14.1 Burets exhibiting chips and cracks will not be used in the laboratory and will be discarded.
- 14.2 When routinely using the burets in the laboratory to dispense liquids, analysts must record the volume dispensed by the buret plus the corrected value, or adjustment, for that volume.
- 15.0 <u>REFERENCES</u>: None

16.0 FORMS AND DATA SHEETS:

16.1 Calibration of Kimble Class A Buret Form.

16.2 Data Analysis Spreadsheet

Calibration of Kimble Class A Buret OPP Microbiology Laboratory

Date/Initials Temperature of Water					
10 mL Buret ID.					
Volume X (mL Buret)	Weight Y (gm)				
2 ml					
4 mL					
6 mL					
8 mL					
10 mL					
50 mL Buret ID					
Volume X (mL Buret)	Weight Y (gm)				
10 mL					
20 mL					
30 mL					
40 mL					
50 mL					

The corrected value for any volume (y) dispensed by the buret is obtained by inserting the volume reading (in mL) on the buret into the following equation: y = mx + b; where y = corrected volume, m = slope (x variable 1 coefficient), x = volume reading on buret, and b = y-intercept (intercept coefficient). The numbers are rounded to the nearest tenth of an mL.

Example of Regression Analysis Spreadsheet

Buret Regression Analysis

Calibration Date: 04/17/04
Analyst Name: J. Smith
Temperature of water: 23°C
Thermometer #: #12345
Buret Volume: 10 mL
Buret ID: #1

<u>Vol. X</u>	<u>Vol. Y</u>
2	1.99465
4	4.01910
6	6.01814
8	8.01560
10	10.00170

Corrected Y Value

V ol. X = 2 mL	y = 2.0
V ol. X = 4 mL	y = 4.0
V ol. X = 6 mL	y = 6.0
Vol. X = 8 mL	y = 8.0
V ol. X = 10 mL	y = 10.0

To determine corrected y value: y = mx + b

SUMMARY OUTPUT

Regression Statistics							
Multiple R	0.999994087						
R Square	0.999988173						
Adjusted R Square	0.999984231						
Standard Error	0.012564167						
Observations	5						

ANOVA

	df	SS	MS	F	Significance F
Regression	1	40.04241124	40.04241124	253660.5	1.72618E-08
Residual	3	0.000473575	0.000157858		
Tota1	4	40.04288481			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0.006658	0.01317741	0.505258638	0.648159	-0.035278398	0.048594398	-0.035278398	0.048594398
X Variable 1	1.00053	0.001986569	503.647183	1.73E-08	0.99420785	1.00685215	0.99420785	1.00685215